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Abstract: In a changing world, plants are exposed to ever changing environmental conditions. Since plants cannot escape from their environment, they require extra flexibility to adapt themselves to stressful conditions, such as drought and salt stresses. One of these adaptations includes the vacuolar accumulation of fructans (fructose-based oligo-and polysaccharides) and anthocyanins (a type of glucosylated flavonoids), both involved in biotic and abiotic stress responses. As part of the recently emerging concept of “sweet immunity” leading to improved tolerance to both abiotic and biotic stresses, possible synergisms between these two types of biomolecules are discussed. Moreover, sucrose-specific signaling pathways stimulate both the production of fructans and anthocyanins, with a central role for MYB-type of transcription factors and a strict dependence on Ca^{2+} . This review summarizes recent insights in these signal transduction pathways and their cross-talks with hormonal signaling pathways, with focus on the stress hormone abscisic acid. Although most of the current research is dedicated on the model plant *Arabidopsis thaliana*, we also focus on wheat as an example of an economically important crop that is able to accumulate both fructans and anthocyanins under various stresses.

Keywords: Anthocyanin, fructan, MYB, stress, sucrose, sugar signaling

1 **1. Introduction**

2
3 In large parts of the world plant production and crop yields are limited due to abiotic
4 stresses including drought, heat, cold, freezing and soil salinity. Abiotic stresses can also
5 make crops more vulnerable to biotic stresses (Shahbaz and Ashraf, 2013). Together, all
6 these stresses seriously compromise food and biomass production. In our changing world,
7 it is important to expand our agricultural systems to drier and saline lands to meet
8 increased food demands, especially in developing countries (Shinozaki and Yamaguchi-
9 Shinozaki, 1999; Huang et al., 2012; Koyoro et al. 2012; Krasensky and Jonak, 2012).

10
11 Among all these stresses, salt stress is certainly one of the most prominent ones causing
12 osmotic and ionic stresses at the cellular level. In general, few crop species are adapted to
13 the presence of high salt levels (Igartua, 1995; Hollington, 1998; Sankar et al., 2011;
14 Radić et al., 2013). In general, plant responses to high salinity are rather complicated,
15 including multiple processes and mechanisms. Extreme salt levels lead to so-called
16 hyperosmotic stress associated with ionic imbalances caused by the increase of toxic ions
17 such as Na^+ and Cl^- . Moreover, salt stress has an unfavorable effect on mineral
18 homeostasis, especially Ca^{2+} and K^+ homeostasis (Isayenkov, 2012; Krasensky and
19 Jonak, 2012). Typically, salt stress enhances abscisic acid (ABA) levels, stimulating
20 stomatal closure which is also associated with increased levels of Reactive Oxygen
21 Species (ROS) and nitrogen oxide (NO) (Allen et al., 1999; Du et al., 2013; Iqbal and
22 Ashraf, 2013). Some of these reactive species trigger signaling pathways that may lead to
23 differential gene expression and (partial) stress adaptation (Ding et al., 2010).

24
25 Wheat is one of the most abundant food crops, which is attacked by numerous viral,
26 bacterial and fungal pathogens and also by insects and nematode pests. An important
27 challenge in current molecular biology of wheat and other crops is to decipher how
28 thousands of genes are differentially expressed under biotic and abiotic stresses, aiming
29 to overcome the detrimental effects of these stresses (Park et al., 2001; Zhu, 2001;
30 Nakashima et al., 2007; Rahaie et al., 2013). Transcription factors (TFs) regulate the
31 expression of target genes by specifically binding to the *cis-acting* elements of (an array
32 of) genes. TFs are classified into different families based on differences in the DNA-

1 binding domains and overall 3D structure (Agarwal et al., 2012; Zhang et al., 2012).
2 Well-known stress-related TF families include the MYB, DREB, NAC, and WRKY types
3 (Singh et al., 2002; Huang et al., 2012). Several studies identified tolerance genes and
4 investigated the TFs involved in wheat salt tolerance (Lee et al., 2007; Ding et al., 2009;
5 Mondini et al., 2012; Zhang et al., 2012). More in particular, plant-specific NACs (NAM,
6 ATAF1, ATAF2 and CUC2) constitute a major TF family with well-known roles in plant
7 developmental programs (Singh et al., 2002; Guo et al., 2004; Huang et al., 2012) and
8 stress responses (Nakashima et al., 2012; Puranik et al., 2012).

9
10 Plants as photo-autotrophic organisms depend on photosynthesis, i.e. the fixation of
11 carbon from carbon dioxide into simple sugar molecules, with the help of sunlight. Next
12 to their role as a carbon and energy source, sugars have acquired important regulatory
13 functions in different organisms early in evolution (Rook and Bevan, 2003). Generally, in
14 higher plants, high sugar levels stimulate expression of genes involved in sink function,
15 such as growth, storage of proteins and biosynthesis of starch (polymers of glucose: Glc)
16 and fructans (polymers of fructose: Fru) (Gupta and Kaur, 2005). In contrast, low sugar
17 levels promote photosynthesis and mobilization of energy reserves, such as breakdown of
18 storage starch or lipids. Next to the total amounts of the small water-soluble
19 carbohydrates Glc, Fru and sucrose (Suc), it seems that the relative proportions of these
20 sugars are important; Glc, Fru and Suc-specific signaling pathways have now been
21 described in plants (Bolouri Moghaddam and Van den Ende, 2013a). Invertases and other
22 Suc-splitting enzymes (e.g. sucrose synthase or SuSy) can have a drastic impact on these
23 different sugar signaling pathways.

24
25 Sugar signaling events include sugar sensing, signal transduction and target gene
26 expression (Goddijn and Smeekens, 1998; Gupta and Kaur, 2005; Rosenquist, 2007). So
27 far, hexokinase (HXK) remains the only well-established sugar sensor in plants,
28 specifically sensing Glc (Moore et al., 2003). However, a Suc-specific signaling pathway
29 seems to be used in many processes including sugar-mediated feedback repression of
30 photosynthesis genes and induction of fructan and starch biosynthetic genes (Vijn and
31 Smeekens, 1999; Rolland et al., 2002). Trehalose 6-phosphate (T6P), synthesized from

1 G6P and UDP-glucose (UDPGlc) by T6P synthase (TPS), is emerging as a central
2 regulator in this Suc-specific pathway (Zhang et al., 2009a). T6P is a direct precursor of
3 the trehalose disaccharide, a well-known stress protectant (Bolouri-Moghaddam et al.,
4 2010).

5
6 Increased proline levels, a widely distributed multifunctional osmoprotectant (Szabados
7 and Savouré, 2010) are often associated with increased Suc levels, demonstrating that
8 proline synthesis also depends on Suc-specific signaling events (Hanson et al., 2008).
9 Accordingly, proline, Suc and fructan levels increased together in salt-stressed wheat,
10 reaching higher levels in salt-tolerant cultivars as compared to a salt-sensitive ones (Kafi
11 et al., 2003). Free proline levels dramatically increased with high salt treatments in
12 *Thellungiella* (Ghars et al., 2012; Wang et al., 2013). In *Arabidopsis*, soluble sugars,
13 anthocyanins (see below) and proline increase together under drought stress (Sperdouli
14 and Moustakas, 2012).

15
16 Plants use a wide array of natural products that often accumulate within vacuoles. Many
17 of those play an essential role in defense responses against herbivores and pathogens.
18 Flavonoids are well-known secondary metabolites, harboring the anthocyanins as typical
19 water-soluble vacuolar compounds (Neilson et al., 2013). Another class of water-soluble
20 vacuolar compounds with anti-stress properties are fructans, occurring in about 15% of
21 flowering plants (Hendry, 1993). Different types of linear and branched fructans can be
22 discerned (Di Bartolomeo et al., 2012). Because of their economic importance, wheat,
23 barley and *Lolium* sp. are among the best-studied fructan accumulators. Wheat plants
24 accumulate branched graminan- (β 2,6 and β 2,1 linkages) and linear levan-type (β 2,6
25 linkages) fructans, especially under stress conditions (Yoshida et al., 2007). Suc
26 stimulates fructan synthesis as a substrate and as an inducer of fructan biosynthetic genes
27 (Müller et al., 2000). Increased Suc and anthocyanin levels occur when wheat leaves are
28 subjected to UV-B (Pradhan et al., 2008; Lv et al., 2013). Some wheat accessions (blue
29 and purple wheat) also accumulate anthocyanins in their grains, which is interesting for
30 food applications (Kniewel et al., 2009). Both fructans and anthocyanins were proposed to
31 play a role in vacuolar antioxidant mechanisms, contributing to cellular ROS homeostasis

(Van den Ende and Valluru, 2009; Bolouri-Moghaddam et al., 2010). While the strong antioxidant capacities of anthocyanins are known since long (Shinozaki and Yamaguchi-Shinozaki, 1999; Gould et al., 2002; Nagata et al., 2003), the ROS scavenging properties of fructans and other soluble plant sugars were only recently recognized (Stoyanova et al., 2011; Hernandez-Marin and Martínez, 2012; Keunen et al., 2013; Peshev et al., 2013).

This review summarizes recent insights on how vacuolar fructans and anthocyanins might contribute to abiotic and biotic stress responses, how sugar signaling and stress hormone pathways might be involved, and how stress-related TFs could influence (or fit into) such signaling pathways. Focus will be on wheat as an example of an economically important crop that is able to accumulate both fructans and anthocyanins under stress. Drought and salt stresses are major problems during wheat grain production (Joudi et al., 2012; Munns et al., 2012).

2. Fructan metabolism in wheat under stress

Fructans are the major storage carbohydrates in temperate grasses and cereals, while starch is often only present at low levels (Chalmers et al., 2005). Fructans play a role in freezing (Yoshida et al., 1998; Kawakami and Yoshida, 2002), drought (Pilon-Smits et al., 1995; Vijn and Smeekens, 1999; Hinch et al., 2000, 2002; Joudi et al., 2012) and salt tolerance (Kerepesi et al., 2002). Plants accumulate fructans for several reasons including their high solubility in water, their resistance to crystallization at subzero temperatures and the fact that the fructan biosynthetic machinery still functions normally under cold stress (Livingston et al., 2009; Krasensky and Jonak, 2012). Furthermore, fructans can stabilize membranes (Vereyken et al., 2001; Hinch et al., 2002; Valluru and Van den Ende, 2008) by inserting at least part of the polysaccharide into the lipid head group region of the membrane (Livingston et al., 2009). However, also Suc and trehalose (Tre) have such membrane stabilizing properties, but probably higher concentrations are needed to reach the same effects (Van den Ende and Valluru, 2009).

Fructans temporarily accumulate in stems and young kernels of wheat (Van den Ende et al., 2003, 2011) and in reproductive organs (Ji et al., 2010). When Suc, the major transport compound in most plants, arrives in such tissues, it can be degraded by Suc splitting enzymes (invertase, SuSy) to sustain growth and/or it can be polymerized into fructans. Suc is the essential substrate for fructan synthesis in wheat by the sequential action of 3 fructosyltransferases (FTs), including 1-SST (sucrose: sucrose 1-fructosyltransferase), 1-FFT (fructan: fructan 1-fructosyltransferase) and 6-SFT (sucrose: fructan 6-fructosyltransferase). Fructan hydrolysis occurs by different types of FEHs (fructan exohydrolases) including 1-FEHs (preferentially degrading β 2,1 linkages) and 6-FEHs (preferentially degrading β 2,6 linkages)(Van den Ende et al., 2003; Kawakami and Yoshida, 2005). 1-SST and 6-SFT are involved in fructan synthesis during cold hardening (Kawakami and Yoshida, 2002), while 6-SFT and 1-FFT play a role in further elongating branched graminans. FTs evolved from vacuolar invertases, while FEHs evolved from cell wall invertases within the family 32 of Glycoside Hydrolases (GH32) (Lasseur et al., 2009; Le Roy et al., 2013). They typically contain highly conserved consensus amino acid motifs including the β -fructosidase motif (DPN) and the RDP and EC motifs harboring the three crucial acidic amino acids in the active site (Van den Ende et al., 2009).

3. Fructans and sweet immunity

Fructans are key compounds within the water soluble carbohydrate (WSC) fraction in wheat involved in osmoregulation and contributing to stress tolerance (Ruuska et al., 2006; Xue et al., 2011, 2012; Joudi et al., 2012). A prolonged cold hardening (-3°C) of winter wheat plants showed a higher expression of both defense-related TFs and genes together with fructan biosynthetic genes, suggesting that fructans might play a role both in biotic and abiotic stress responses (Gaudet et al., 2011). Accordingly, spraying a fructan containing preparation from burdock (*Arctium lappa*) (termed burdock fructosyl oligosaccharide or BFO) on tobacco leaves increased the resistance against tobacco mosaic virus (TMV) which was correlated with an increased expression of a number of defense genes (Wang et al., 2009). These authors proposed the involvement of a salicylic acid (SA)-dependent signaling pathway in the process. Further, a pretreatment with BFO

1 reduced the lesions caused by *Colletotrichum orbiculare* by 56.8%. Additionally, the
2 amount of lignin and the activities of defense-related enzymes (peroxidase, superoxide
3 dismutase, polyphenoloxidase and β -1,3-glucanase) significantly increased in leaves
4 pretreated with BFO and during the subsequent inoculation (Zhang et al., 2009b).

5
6 Application of BFO on different fruits also aimed at controlling postharvest diseases (Sun
7 et al., 2013). In tomato fruit, BFO and chitosan oligosaccharides effectively inhibited
8 natural postharvest diseases and reduced disease incidence after inoculation with *Botrytis*
9 *cinerea*. Furthermore, BFO increased the mRNA level of genes encoding pathogenesis-
10 related proteins (PRs), such as PR-1a, PR-2a (extracellular β -1,3-glucanase), PR-2b
11 (intracellular β -1,3-glucanase), PR-3a (extracellular chitinase) and PR-3b (intracellular
12 chitinase), and induced the mRNA accumulation of the phenylalanine ammonialyase
13 (PAL) gene (Zhang et al., 2009b). All these observations strongly suggest that fructans,
14 similar to Suc (Gómez-Ariza et al., 2007) and an array of different oligosaccharides
15 (Bolouri Moghaddam and Van den Ende, 2012; 2013a) may be involved in sweet
16 “priming” processes, stimulating the plant’s immune system, and counteracting both
17 biotic and abiotic stresses.

18
19 After applying Alginate Oligosaccharides (AOS) on wheat, antioxidative enzymes
20 activities were enhanced and the malondialdehyde (MDA) content was reduced.
21 Additionally, the drought resistant related genes targeted by the ABA signaling pathway
22 (see also below), such as the late embryogenesis abundant protein 1 gene (LEA1), the
23 psbA gene, the Sucrose non-fermenting 1-related protein kinase 2 gene (SnRK2) and the
24 Pyrroline-5-Carboxylate Synthetase gene (P5CS) were up-regulated by AOS (Liu et al.,
25 2013). AOS can also stimulate root development in wheat, with a role for NO signaling
26 mechanisms (Zhang et al., 2013). Taken together, these observations show that plant and
27 algal-derived oligosaccharides can strongly influence plant development and stress
28 responses.

31 **4. Fructans as putative vacuolar antioxidants or stress signals**

1
2 Environmental factors such as drought, salinity, ozone and high or low temperatures limit
3 CO₂ fixation, reducing the NADP⁺ regeneration by the Calvin cycle. As a consequence,
4 the photosynthetic electron transport chain gets over-reduced, producing superoxide
5 radicals and singlet oxygen in chloroplasts (Hare et al., 1998; Shao et al., 2008),
6 damaging membranes, DNA and proteins and inhibiting *de novo* protein synthesis
7 (Murata et al., 2007), potentially leading to ageing or cell death (Van Breusegem and Dat,
8 2006; Marchi et al., 2012).

9
10 Fructans are probably able to counteract oxidative stresses, since introduction of plant
11 fructan biosynthesis genes in non-fructan accumulators results in stress tolerance (Li et
12 al., 2007; Kawakami et al., 2008; Bie et al., 2012; Van Arkel et al., 2013). Transgenic
13 tobacco with *sacB*, a gene encoding a bacterial levansucrase, increased fructan-
14 accumulation under drought stress (Pilon-Smits et al., 1995), as well as under salt stress
15 and freezing conditions (Doltchinkova et al., 2013). It was particularly striking that
16 introduction of the wheat 1-SST or 6-SFT genes in rice resulted in improved chilling
17 tolerance at the seedling stage (Kawakami et al., 2008). Similar results were obtained
18 after overexpression of genes involved in the biosynthesis pathway of Raffinose Family
19 Oligosaccharides (RFOs: α -galactosyl extensions of Suc) (Nishizawa et al., 2008). It was
20 hypothesized that fructans and RFOs might function as transportable stress signals (Van
21 den Ende and Valluru, 2009; Bolouri-Moghaddam et al., 2010). Also, it has been
22 suggested that galactinol, RFOs and fructans might be essential in scavenging hydroxyl
23 radicals in the vicinity of membranes *in vivo* since such reactions could be demonstrated
24 *in vitro* (Nishizawa et al., 2008; Peshev et al., 2013). While fructans are ideal candidates
25 for tonoplast stabilization and protection, RFOs such as raffinose seem to target the
26 chloroplast thylakoid membranes (Knaupp et al., 2011; Nägele and Heyer, 2013). Indeed,
27 raffinose can be imported in chloroplasts (Schneider and Keller, 2009). Accordingly,
28 improved thylakoid membrane stability and chloroplast integrity under stress was
29 observed in transgenic plants accumulating fructans and other osmolytes (Doltchinkova
30 et al., 2013). The exact chemical reactions between fructans, raffinose and the hydroxyl
31 radical are now better understood. This reaction leads to the generation of a sugar radical

1 and it is a matter of speculation how sugars are then regenerated from these sugar
2 radicals. Likely, phenolic compounds such as anthocyanins are involved in these
3 processes, while final reduction of the resulting oxidized phenolic compounds is
4 accomplished by ascorbate or glutathione which needs to be imported into the vacuole
5 (Peshev et al., 2013). It can be speculated that similar reactions might occur in the human
6 body as well (Van den Ende et al., 2011; Dahech et al., 2013).

7 8 **5. Sugar and hormone signaling influencing carbohydrate metabolism under** 9 **stress**

10
11 Salt stress is particularly detrimental for crop plant growth and development (Inan et al.,
12 2004). To protect themselves against the salinity condition (Shahbaz and Ashraf, 2013),
13 plants adapt by changing their ion homeostasis and adjusting their growth. Soluble sugars
14 and proline are important osmolytes that accumulate in plants under salt stress, playing
15 vital roles in integrating environmental signals (Volkov and Amtmann, 2006). Nitrogen
16 limitation and high-intensity light typically stimulate sugar accumulation resulting in
17 direct (e.g. membrane stabilization, ROS scavenging) and indirect (e.g. signaling) effects
18 (Bolouri-Moghaddam et al., 2010). Transgenic cotton plants with a vacuolar H⁺-PPase
19 gene are more resistant to NaCl than non-transgenic plants, and this may be attributed to
20 their enhanced sugar levels when exposed to salt stress (Lv et al., 2008). High levels of
21 soluble sugars, in particular Glc, Fru, mannitol and several amino acids, were detected in
22 the halophyte *Thellungiella* exposed to high salt levels (Wang et al., 2013).

23
24 In many cases Suc can trigger effects in plants that cannot be triggered by using
25 equimolar concentrations of Glc plus Fru. Such Suc-specific signaling pathways are
26 involved in the synthesis of two types of vacuolar compounds: anthocyanins (Teng et al.,
27 2005; Solfanelli et al., 2006) and fructans (Martínez-Noël et al., 2009, 2010). Since Suc is
28 both essential for the induction of FT genes and as a substrate for the FT enzymes
29 synthesizing fructans, it is clear that the actual Suc levels greatly determine potential
30 fructan accumulation. The actual Suc levels are determined by the balance between Suc
31 biosynthesis (SPS/SPP) and Suc degradation (invertases, SuSy). These genes are
32 influenced by many plant hormones and by the circadian clock as recently reviewed

1 (Bolouri Moghaddam and Van den Ende, 2013b). Thus, hormones and the clock can
2 indirectly influence the potential of fructan synthesis through its effect on Suc
3 metabolism. It is also clear that the activity of Suc splitting enzymes and FEHs can
4 greatly influence cellular Hex/Suc ratios, promoting hexose signaling which is usually
5 associated with sink growth.

6
7 In *Arabidopsis* seedlings, extensive crosstalk occurs between Glc and ABA signaling.
8 Glc activates both ABA synthesis and signaling and ABA and Glc signaling are
9 antagonistic to ethylene signaling (Arroyo et al., 2003; Rolland et al., 2006). Fru-specific
10 FSQ6 downstream signaling interacts with ABA- and ethylene-signaling pathways,
11 similar to HXK1-dependent Glc signaling (Li et al., 2011). Similar extensive crosstalks
12 between Suc and hormonal signaling pathways are expected.

13
14 To illustrate the particular importance of ABA as a stress hormone in the sugar signaling
15 context, it should be noted that comparisons of transcriptomes of *Arabidopsis* and rice
16 exposed to ABA and various abiotic stresses have shown drastic changes affecting 5–
17 10% of the genome (Shinozaki et al., 2003; Nakashima et al., 2009). ABA also plays a
18 vital role in the regulation of the key enzymes involved in fructan and Suc metabolism in
19 wheat (Yang et al., 2004). ABA can substitute for a low temperature stimulus, provided
20 there is also an adequate supply of sugars (Gusta et al., 2005). ABA concentrations
21 increase under drought and this is significantly correlated with increased SPS and FEH
22 activities in wheat stems, while water stress reduced the 1-SST activities. In contrast,
23 application of fluridone, an inhibitor of ABA synthesis, significantly reduced the
24 activities of FEH, SPS and acid invertase, carbon remobilization and grain weight (Yang
25 et al., 2004). Gene transcripts for 1-SST, 1-FFT and 6-SFT and 6-FEH increased, while
26 transcripts for 1-FEH decreased, in Suc-fed wheat stems compared to controls. However,
27 opposite trends were observed in wheat stems fed with ABA (Ruuska et al., 2008). In
28 maize leaves, ABA is known to be a powerful enhancer of the IVR2 vacuolar invertase
29 activity and expression (Trouverie et al., 2003).

Protein 2C and 2A play vital roles in the early signaling pathway of ABA (Hauser et al., 2011; Lee and Luan, 2012). Many stress-inducible protein kinase families are activated by ABA and other stress signals. These include mitogen-activated protein kinases (MAPKs) (Wrzaczek and Hirt, 2001), calcium-dependent protein kinases (CDPKs) (Ludwig et al., 2004), and sucrose non-fermenting 1 (SNF1)- related protein kinases (SnRKs) (Fujii et al. 2011; Tian et al., 2013). Three subfamilies can be discerned, SnRK1, SnRK2 and SnRK3, with a total of 38 members in the model plant *Arabidopsis* (Halford and Hey, 2009). Stress responses typically involve the synthesis of dehydrins and “late embryogenesis abundant” (LEA) proteins, and in addition important enzymes for the synthesis of osmoprotectants such as proline and sugars. DREBs (dehydration-responsive element-binding proteins), AREBs (ABA-responsive element-binding proteins) and NAC proteins are typical TFs that are involved in these responses. An SnRK2 homologue in wheat (*PKAB1* or *TaW55*) is typically induced by ABA and hyperosmotic stress and seems to be involved in the response to multiple environmental stress (Xu et al., 2009). However, not all SnRK2 types are activated by ABA (Kulik et al., 2011). A plasma membrane-associated NAC protein (NTL6) gets activated after C-terminal processing and then acts as a TF for the induction of PR genes under cold (Seo et al., 2010). It was suggested that NTL6 phosphorylation by SnRK2.8 may be needed for its nuclear import and regulatory role during drought tolerance (Kim et al., 2012). In *Arabidopsis*, a Ca^{2+} -dependent Salt Overly Sensitive (SOS) pathway, with different SOS SnRK3 protein kinases involved, fulfills a major role in salt stress responses (Liu et al., 2000; Ji et al., 2013). SnRK3 is vital for Na^+ and K^+ homeostasis and abiotic stress tolerance (Liu et al., 2000). Similar to other SnRKs, they may function as cross-talk nodes in complex signaling networks (Nolan et al., 2006).

6. Anthocyanin formation and its functions

Anthocyanins, a class of flavonoids, do not only provide color to flowers and fruits, attracting animals for pollen dispersal and pollination. They are believed to provide photoprotection *via* light attenuation and/or antioxidant activity (Hughes et al., 2013 and references therein). Although their function in osmoregulation has been suggested, their

1 actual concentration is likely too low to fulfill such role (Hughes et al., 2013). They are
2 recently emerging as putative important regulators of plant development and stress
3 responses (Winkel-Shirley, 2001; Pourcel et al., 2013). Roles for anthocyanins in
4 herbivore protection have been suggested (Close and Beadle, 2003; Cooney et al., 2012),
5 but more experimental verification is necessary.

6
7 The biosynthesis of anthocyanins is mediated by multiple enzymes in the
8 phenylpropanoid pathway. The coding genes of these enzymes are grouped into two
9 classes: early biosynthetic genes [Chalcone synthase (CHS), chalcone isomerase (CHI)
10 and the Flavanone 3 β -hydroxylase (F3H)], and late biosynthetic genes [dihydroflavonol
11 reductase (DFR), anthocyanidin synthase (ANS), and leucoanthocyanidin dioxygenase
12 (LDOX)] (Zhou, et al., 2008; Tanaka et al., 2008). CHS catalyzes the first step in the
13 phenylpropanoid pathway (Feinbaum and Ausubel, 1988). The CHI enzyme, which
14 catalyzes the second step in the pathway (Shirley et al., 1992), utilizes
15 tetrahydroxychalcone as a substrate and converts it to naringenin. Next, F3H
16 hydroxylates flavonones at the 3 position to form dihydroflavonols, which are required
17 for the synthesis of anthocyanidins and flavonols. The next step in the flavonoid pathway
18 is DFR which reduces dihydroflavonols to leucoanthocyanidins. Then ANS, encoding a
19 dioxygenase, produces the substrate for UDPGlc flavonol 3-O-glucosyl transferase,
20 glycosylating anthocyanidins and flavonols on the 3 position (Clegg and Durbin, 2000;
21 Pourcel et al., 2013).

22
23 Anthocyanins also display a variety of biological activities. Antiinflammatory (Dai et al.,
24 2007), anticarcinogenic (Neto, 2007) and antimicrobial properties have been proposed
25 (Cisowska et al., 2010). Moreover, anthocyanins play an important role in plant stress
26 responses as strong antioxidant molecules protecting plants from ROS damage (Gould et
27 al. 2002; Nagata et al., 2003). Pourcel et al. (2013) hypothesized that (some) flavonoids
28 function as buffering or even signal molecules to adjust responses induced by biotic and
29 abiotic stress conditions. Dihydrochalcones (mainly phloridzin, sieboldin, trilobatin and
30 phloretin) represent the major flavonoid subgroup in apple green tissues. It was proposed

1 that they contribute to basal defense. In particular phloretin possesses a broad-range of
2 bactericidal and fungicidal characteristics (Gaucher et al., 2013).

3
4 In another stress experiment, the intraspecific responses of 12 winter wheat cultivars to
5 different doses of ultraviolet-B (UV-B) radiation were analyzed and compared. It was
6 found that the response indexes of plant anthocyanin were significantly correlated with
7 the cumulative stress response indexes, indicating that anthocyanins are vital to UV-B
8 tolerance (Lv et al., 2013). Anthocyanins were also found to be major contributor to the
9 total antioxidant activity from blue wheat extracts, which are popular in food applications
10 (Tyl and Bunzel, 2012). Low-temperature associated soluble carbohydrate accumulations
11 are commonly associated with anthocyanin coloration, attenuation of growth and cold
12 adaptation in cool-season grasses. Therefore, anthocyanin coloration was even proposed
13 as a useful phenotypic marker for soluble carbohydrate accumulation (Hu et al., 2002).
14 Suc signaling processes and increased isoflavonoid levels lead to increased defense
15 responses against *Fusarium* in lupine (Morkunas et al., 2005, 2011, 2013).
16 Overexpressing of a tea DFR and ANR in tobacco led to higher flavonoid levels, a higher
17 antioxidant capacity and better resistance against infestation by a tobacco leaf cutworm
18 *Spodoptera litura* (Kumar et al., 2013). This suggests that anthocyanins themselves
19 somehow contribute to multistress responses. Alternatively, or additionally, uncoupled
20 from their catalytic activity, DFR and ANR may also function as regulatory proteins
21 involved in the stimulation of pathways that counteract multiple stresses, and these are
22 exciting avenues for future research. Similarly, it was recently demonstrated that
23 glyceraldehyde 3 phosphate dehydrogenase not only functions as a glycolytic enzyme but
24 also as a key regulatory player to determine the function of T-cells as crucial players in
25 immunity responses (Chang et al., 2013).

26 27 **7. Regulation of anthocyanin and fructan formation**

28
29 In Arabidopsis, a regulatory complex consisting of R2R3-MYB, basic helix-loop-helix
30 and WD-repeat proteins controls anthocyanin biosynthesis (Ramsay and Glover, 2005).
31 The PAP1/MYB75, PAP2/MYB90, MYB113 and MYB114 TFs provide the R2R3-MYB

function (Borevitz et al., 2000; Gonzalez et al., 2008). Two MYB genes, *PAP1/MYB75* and *PAP2/MYB90*, are involved in the regulation of anthocyanin synthesis (Borevitz et al., 2000). A Suc-specific signaling pathway induces the expression of *PAP1/MYB75* and, as a consequence, the anthocyanin synthesis pathway is stimulated (Kranz et al., 1998; Teng et al., 2005). Bhargava et al. (2010) reported that a MYB75 loss-of-function mutation (*myb75-1*) resulted in increased cell wall thickness, suggesting that MYB75 acts as a repressor of the lignin branch of the phenylpropanoid pathway in Arabidopsis. *MYB75* overexpression results in red-colored leaves and pink roots and flowers in Arabidopsis. Similar phenotypes were found in transgenic tobacco plants (purple leaves, stems, roots, and flowers; Borevitz et al., 2000) and in transgenic tomato plants (red coloration in the veins, rachis, and petioles, and a reddish-purple pigmentation in stems, flowers and fruits; Zuluaga et al., 2008). Furthermore, overexpression of *MYB75* in canola resulted in purple plants (Li et al., 2010). By overexpressing the apple MYB10 TF, apple trees with highly pigmented red foliage were obtained (Markwick et al., 2013).

MYB75 and *MYB90* are upregulated under low nitrogen (Fig. 1; Lea et al., 2007). *CHS*, *CHI*, *F3H* and *DFR* are induced under limiting nitrate conditions, and the generated anthocyanins might protect plants photo-damage resulting from low nitrogen levels (Peng et al., 2007). The induction of rapid wound-responsive genes was significantly delayed and reduced when naringenin was added to wounded *CHI* mutant plants, indicating that flavonoids may act as general modulators of stress responses (Fig. 1; Oh et al., 2011; Pourcel et al., 2013). HY5, a downstream component of phytochrome (PHY), cryptochrome (CRY), and UV-B (UVR8) photoreceptor-mediated light signaling regulate *MYB75* expression via direct binding to G- and ACE-boxes in the promoter region, which suggests bifurcate regulation of anthocyanin biosynthesis by HY5 via transcriptional activation of *MYB75* (Fig. 1; Shin et al., 2013).

As mentioned before, anthocyanin synthesis is under control of a Suc-specific pathway in Arabidopsis but extensive crosstalk with hormonal signaling pathways can be expected, especially with ABA. ABA causes a hypersensitive response leading to anthocyanin accumulation in rice (Zhou et al., 2009), maize (Hattori et al., 1992; Kim et al., 2006) and

1 in grapes (Mori et al., 2005). In Arabidopsis seedlings, ABA alone induced the central TF
2 *MYB90* (Fig. 1), but in combination with Suc a synergistic effect was observed,
3 significantly increasing the transcript levels of the biosynthetic genes involved in
4 anthocyanin accumulation such as *CHS*, *C4H*, *DFR*, *LDOX* and *UF3GT* (Loreti et al.,
5 2008). In maize, no accumulation of anthocyanin was noticed in viviparous-1 (*vp1*), an
6 ABA insensitive mutant inhibiting the expression of the *C1* gene (a MYB TF similar to
7 *MYB75* and *MYB90*) (McCarty et al., 1989; Hattori et al., 1992). Sugar signaling
8 associated with anthocyanin accumulation is intimately linked to several signaling
9 pathways such as those triggered by light, ABA, cytokinin, ethylene and JA , which
10 might be interconnected with Ca^{2+} signaling by mechanisms that require further research
11 (Vitrac et al., 2000).

12
13 In wheat and other grasses, fructan biosynthesis can be easily induced in the light with
14 exogenous Suc (Müller et al., 2000; Maleux and Van den Ende, 2007). As mentioned
15 above, the expression of FT genes is controlled by a Suc-specific pathway (Martínez-
16 Noël et al., 2006; Ritsema et al., 2009). Similar to the Arabidopsis anthocyanin signaling
17 pathway where *MYB75* and *MYB90* take a central position, 3 MYB TFs (*TaMYB13-1*,
18 *TaMYB13-2* and *TaMYB13-3*) were recently identified that play a role in the pathway
19 leading to fructan synthesis in wheat (Fig. 1). These MYBs bind to the promoter region
20 of wheat 1-SST and 6-SFT genes and stimulate their expression (Xue et al., 2011).
21 Before, a WRKY-type *SUSIBA1* TF was described acting as a repressor of the 1-SST
22 and 6-SFT genes in barley (Fig. 1) (Rosenquist, 2007). It is not yet known whether cereal
23 *SUSIBA* and *MYB13* type TFs can influence each other. Furthermore, it will be
24 interesting to investigate whether SnRK1 counterparts in wheat are controlling the
25 *TaMYB13* TFs (Fig. 1).

26
27 Stress-derived energy deficiency activates SnRK1 protein kinases (Baena-González et al.,
28 2007; Jossier et al., 2009), leading to a major transcriptional reprogramming, partly via
29 TFs and partly via miRNAs (Confraria et al., 2013). Besides their well-known critical
30 role in maintaining energy homeostasis, SnRK1s can also critically influence stress-
31 inducible gene expression and the induction of stress tolerance (Cho et al., 2012;). It has

1 been shown that SnRK1 interacts with myoinositol polyphosphate 5-phosphatase
2 (5PTase13), linking inositol, sugar, and stress signaling (Ananieva et al., 2008). The
3 KIN10/11 subunits of SnRK1 also interact with a NAC domain TF, ATAF1/ANAC002,
4 with well-recognized roles in plant development and stress responses (Kleinow et al.,
5 2009). KIN10 overexpressors confer an ABA-hypersensitive phenotype, suggesting that
6 this SnRK1 links these two signaling pathways (Jossier et al., 2009).

7
8 While SnRK2 was recently recognized as an integral part of the ABA-signaling pathway
9 (Sreenivasulu et al., 2012), it is not yet clear whether SnRK1 and its inhibitor, T6P, make
10 up an integral part of a Suc-specific signaling pathway leading to anthocyanin
11 accumulation in Arabidopsis (Fig. 1). Since no Suc sensor could be identified so far, one
12 possible hypothesis is that the Suc signal might be modified into a T6P signal, inhibiting
13 SnRK1 (Zhang et al., 2009a), which in itself inhibits MYB75 (Baena-González et al.,
14 2007) for stimulating the synthesis of anthocyanins. Accordingly, good correlations were
15 found between Suc and T6P levels, both in Arabidopsis (O'Hara et al., 2013; Nunes et
16 al., 2013b) and in wheat tissues (Martínez-Barajas et al., 2011). Moreover,
17 overexpression of TPS led to increased trehalose and proline levels and increased
18 tolerance to abiotic stresses (Li et al., 2011). Besides T6P, also glucose 6-phosphate
19 (G6P) and glucose 1-phosphate (G1P) can inhibit SnRK1 (Fig. 1), but to a lesser extent
20 as compared to T6P (Nunes et al., 2013a).

21
22 Calcineurin B-like (CBL) proteins are Ca^{2+} sensors and well-known modulators of
23 abiotic stress responses. It was found that the CBL1 protein interacts physically with
24 KIN β 1, the regulatory β subunit of the SnRK1 complex with its central role in sugar
25 signaling (Fig. 1). It was proposed that CBL1 and KIN β 1 form a complex that is attached
26 to the plasma membrane (Fig. 1; Li et al., 2013b). On the one hand, overexpression of
27 KIN β 1 caused a disturbed development with less expanded cotyledons and no
28 development of true leaves in the presence of sugar (Li et al., 2009). On the other hand,
29 *cbl1* displays a sugar-sensitive phenotype similar to the one observed in KIN β 1
30 overexpressors (Li et al., 2013b). Thus, CBL1 and SnRK1 may exert opposite functions
31 in sugar signaling (Fig. 1), but this requires further experimental verification.

8. A role for Ca^{2+} in the pathways leading to fructan and anthocyanin synthesis

Overall Ca^{2+} is considered as a second messenger in the signal transduction pathways of hormones and environmental stimuli in plants (Shin et al., 2012). Ca^{2+} signaling processes are considered as one of the earliest events in salt stress signaling. Ca^{2+} antagonists strongly interfere with Suc uptake and anthocyanin accumulation by down-regulating the expression of Suc transporter 1 (*SUC1*) and *MYB75*. Time course analysis of the effect of Ca^{2+} antagonists showed that an early inhibition of Suc-induced sugar uptake led to decreased anthocyanin accumulation, indicating that Ca^{2+} signals play a major role in Suc uptake (Shin et al., 2013).

As already indicated above, CBLs make up a unique group of Ca^{2+} sensors in plants. CBLs regulate CBL-interacting protein kinases (CIPKs). For instance, CIPK7 plays a role in cold responses via its interaction with CBL1 (Huang et al., 2011). Moreover, CBLs participate in salt stress signal transduction pathways to control the influx and efflux of Na^+ (Agarwal and Sheel, 2012). An inhibition of anthocyanin accumulation was observed with two Ca^{2+} channel blockers in grape cell cultures, confirming that sugar-stimulated anthocyanin biosynthesis depends on Ca^{2+} . Therefore, Ca^{2+} , calmodulin and protein kinases/phosphatases are considered as important components of the sugar signaling pathways leading to anthocyanin biosynthesis. Furthermore, ABA applications are known to stimulate the cytosolic Ca^{2+} levels (Allen et al., 1999). Ca^{2+} then regulates the activities of target proteins via Ca^{2+} binding proteins such as calmodulin, on its turn activating CDPKs and other proteins in plant cells (Vitrac et al., 2000).

Intriguingly, Ca^{2+} and NO are involved in both the pathways leading to fructan and anthocyanin formation (Fig. 1) (Martínez-Noël et al., 2006; Tossi et al., 2011; Shin et al., 2012), suggesting that (at least part of) these pathways might be rather similar in both cases. In Arabidopsis, the accumulation of NO is independent of the Nitric Oxide Associated (NOA1) protein, in the presence of Suc (Van Ree et al., 2011). The exogenous application of NO alleviated the negative effects of chilling stress by

stimulating fructan accumulation in winter wheat but not in spring wheat, suggesting variety-specific differences and crosstalk between NO and sugar signaling (Li et al., 2013a).

Conclusion and perspectives

Biotic and abiotic stresses stimulate the accumulation of stress-related metabolites such as vacuolar fructans, anthocyanins and proline. A number of striking synergisms can be detected. First, the synthesis of all these compounds not only depends on Suc as a substrate but also on Suc-specific signaling events. However, the details on the underlying pathway(s) are still unknown and the Suc sensor(s), if existing, remain(s) to be identified. One line of thinking is that the Suc signal is translated into a T6P signal, inhibiting SnRK1, but this requires further verification. Second, both Ca²⁺ and NO are probably involved in the signaling pathways leading to anthocyanin and fructan synthesis. Third, MYB transcription factors fulfil a central role in both signaling pathways. Fourth, as described before for Glc-mediated signaling, there is extensive crosstalk between the Suc-specific signaling pathways and hormone signaling pathways, especially with ABA.

Further research is necessary to understand how these Suc-specific signaling pathways exactly contribute to the recently emerging “sweet immunity” or “sweet priming” concept involved in biotic and abiotic stress responses (Bolouri Moghaddam and Van den Ende, 2012, 2013a). An intriguing hypothesis is that the intermediates or the final products of such Suc-specific signaling pathways (anthocyanins, fructans, proline) function as “secondary” signals under stress, opening a new era of research in plant stress physiology.

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18
19 **Figure legend**

20 Figure 1. Emerging connections between sucrose-specific signaling pathways leading to
21 fructan (wheat) and anthocyanin (Arabidopsis) accumulation under stress.

22
23 SnRK1 might fulfill a central role in such pathways, similar to the role of SnRK2 in ABA
24 signaling. Although it is clear that other kinases (MAPK, CDPK), as well as NO, Ca²⁺
25 and ROS signaling are involved in stress responses, it is not yet clear whether some make
26 up an integral part of sucrose-specific signaling pathways. Alternatively, intimate cross-
27 talk might exist between different stress signaling pathways, with prominent roles for
28 SnRKs at the interface of these pathways. In signaling and metabolic events, positive and
29 negative interactions are provided by dashed black arrows and red “inhibitory” arrows,
30 respectively. Unclear or hypothetical interactions are indicated with a dashed line.
31 TaMYB13 and SUSIBA1 control the expression of fructan biosynthetic genes in a
32 positive and negative way, respectively. TaMYB75/PAP1 and TaMYB90/PAP2 are

central players in the anthocyanin pathway; both are up-regulated under low nitrogen conditions. ABA induces MYB90 in Arabidopsis, while SnRK1 inhibits TaMYB75, which is in itself inhibited by, G1P, G6P and T6P. HY5 also activates MYB75 expression via direct binding to G- and ACE-boxes in the promoter region. It is not completely clear whether fructans and anthocyanins (or proteins involved in their biosynthesis) counteract stresses via direct (ROS scavenging, membrane stabilization, photoprotection) or indirect (e.g. signaling) mechanisms. Although vacuolar fructans and anthocyanins are more considered as abiotic stress modulators, evidence is accumulating that they may be effective in counteracting biotic stresses as well. Therefore, it can be hypothesized that Suc-specific signaling pathways may be central in counteracting multiple stresses in plants through the so-called “sweet immunity” concept (Bolouri Moghaddam and Van den Ende, 2012, 2013a). We refer to the text for more details and for the used abbreviations.

